In vitro biological activity of decoction of Joshanda

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Abstract: Joshanda is a polyherbal product, commonly practicing in inflammation of upper respiratory tract as tea. The present study was conducted to find out its antimicrobial, phytotoxic, leishmanicidal and cytotoxic activities. The decoction of the product showed profound activity against Gram positive tested pathogens especially *S. aureus* 36.5 mm zone of inhibition at 8.0µg/ml. However, it was inactive against *C. albicans*. Closed correlation was observed between two methods in terms of results. It had potent phytotoxic activity (75%). However, it was devoid of any activity leishmanicidal and cytotoxic activity. Phytochemical studies of Joshanda showed the presence of various pharmacologically active groups.

Keywords: Joshanda, decoction, antibacterial, antifungal, phytotoxic, phytochemicals.

INTRODUCTION

Joshanda is a polyherbal formulation of Unani origin (Greco-Arab). It is largely used against the inflammation of the mucous membranes of nose and air passages (Azmi *et al*., 2010). Joshanda is one of the leading house hold remedy against upper respiratory infections, catarrh, cold and flu in Pakistan. Such practice is even common in paediatric age group (Ashraf *et al*., 2010). This polyherbal formulation consists of expectorant, respiratory demulcent and anticatarrhal herbs which assist in relieving the enervating cough. It is also recommended for the treatment of premenstrual syndrome (Akram *et al*., 2011). The effect of the drug on the bronchial smooth muscles in isolated tissues is already explored (Kheterpal *et al*., 1989). It showed potent anti-inflammatory activity in animal studies that were partially mediated through lipoxygenase; strongly augmented by its antioxidant activities (Khan *et al*., 2012a).

In continuation of our previous studies (Khan *et al*., 11a; Khan *et al*., 12b; Khan *et al*., 2012c; Khan *et al*., 2013; Ibrar *et al*., 2012) in which different folk uses of medicinal plant were validated in the light of established experimental paradigm apparently enriches our ethnopharmacology. The current study was designed to explore its effect in various antimicrobial especially those involved in respiratory tract infections, phytotoxic, leishmanicidal and cytotoxic assays.

MATERIAL AND METHODS

Sample Collection

Joshanda (Hamdard Laboratories Waqf Pakistan). was obtained in commercial pack from a herbal medical store in Peshawar. Each commercial packet contained the same composition of plants. 100g of each Joshanda packet contains 3g of *Althaea officinalis*, 9g of *Cordia latifolia*, 5g of *Glycyrrhiza glabra*, 3g of *Malva sylvestris*, 5g of *Onosma bracteatum* 5g of *Viola odorata* and 5g of *Zizyphus Sativa*.

Sample preparation

All the materials from the commercial packets were taken out and ground to powder form using grinding machine. The powdered materials were weighed. The sample was taken in hot water at 70ºC for 24 h to make a decoction. Then it was filtered while hot on Buckner funnel using vacuum pump. The filtrate was centrifuged for 40 min at 5000 rpm to separate out solid particles. The liquid mixture was concentrated using vacuum rotary at 70ºC (Khan *et al*., 2012a). Subsequently it was dried in oven and finally ground to powder using mortar and pistol. The % yield was calculated as 17.3%.

Antimicrobial activities

The antimicrobial activities of Joshanda extract were determined using Kirby-Bauer Disc Diffusion Method (American Society for Microbiology, 1972). One liter of agar gel was prepared by dissolving 28 gm. of agar in one liter of distilled water. The mixture was autoclaved at 120 ºC under 1.5 bar pressure for 15 min. Then this medium was poured in Petri dishes inside laminar hood flow (LHF) already sterilized by wiping with ethyl alcohol. Then this medium was incubated for 24 h at 37ºC to check its purity. Then four strains of gram negative bacteria, *K. pneumonia*, *S. typhi*, *P. aeruginosa* and *E. coli* were streaked on these plates using a sterilized platinum loop. This streaking was done inside Laminar hood flow (LHF), sterilized with ethyl alcohol. Then these Petri

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Table 1: Antibacterial activity of decoction of Joshanda in paper disc method

Bacteria	Zone of Inhibition (ml)				
	Sample concentration in ml				
	A	B	DMSO	Ciprofloxacin	Erythromycin
K. pneumoniae	8.0±0.2	14.5±0.2	−	27±0.02	−
S. typhi	0.00	0.00	−	8±0.01	−
P. aeruginosa	0.00	0.0	−	15±0.02	−
E. coli	0.00	0.00	−	31±0.02	−
S. aureus	33.5±0.1	36.5±0.1	−	−	26.9±0.03
B. subtilus	10.00±0.1	15.5±0.3	−	−	27±0.02
B. cereus	9.0±0.2	15.2±0.1	−	−	25±0.01

Data represent mean ± SEM of three individual studies.

Table 2: Antibacterial activity of decoction of Joshanda in agar well method.

Bacteria	Zone of Inhibition (ml)				
	Sample concentration in ml				
	A	B	DMSO	Ciprofloxacin	Azthromycin
K. pneumoniae	11.0±0.01	16.5±0.2	−	27±0.02	−
S. typhi	0.00	0.00	−	8±0.01	−
P. aeruginosa	0.00	10.0	−	15±0.02	−
E. coli	0.00	0.00	−	31±0.02	−
S. aureus	38.5±0.01	40.5±0.02	−	−	26.9±0.03
B. subtilus	15.5±0.02	17.5±0.01	−	−	27±0.02
B. cerus	12.5±0.02	14.5±0.03	−	−	25±0.01

Data represent mean ± SEM of three individual studies.

dishes containing bacterial strains were incubated for 24 h at 37ºC. After 24 h incubation, the bacteria were transferred from agar gel into a broth medium. Then broth containing bacteria was constantly shaken in a shaker for 18 h. The bacteria from broth were then spread uniformly on agar plates with the help of glass spreader. Now the sample along with standard was inoculated using paper disc (Qadrie *et al*., 2009) and agar well diffusion (Khan *et al*., 2011) methods. After inoculation, these plates were incubated for 24 h at 37ºC. After 24 h inoculation all the plates were checked for their activity.

Microorganisms

The bacterial strains used in the test were *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *B. cereus* (clinical isolate) *E. coli* ATCC 25922, *S. flexeneri* (clinical isolate) *P. aeruginosa* ATCC 27853 *and S. typhi* ATCC 19430. The reference fungal strains were *C. albicans* ATCC 2091.

Phytotoxic assay

Phytotoxic activity of the decoction of Joshanda was evaluated according to our previously reported method (Khan *et al*., 2011b). The medium was prepared by mixing various inorganic constituents in distilled water (100/ml) and the pH was adjusted (5.5 to 6.5) with KOH solution. The medium was then autoclaved at 121°C for 15 min. The samples (30.0/g) dissolved in ethanol (15/ml) served as stock solution. Sterilized 9 flasks, three for each concentration, were inoculated with 1000, 100 and 10 µl of the stock solution to give the final concentration of 1000, 100 and 10 µg/ml respectively. The solvent was allowed to evaporate overnight under sterile condition. To each flask, 20 ml of the medium at a pH of 5.5 to 6.5 was added. Then 10 plants of *L. acquinoctialis* Welv, each containing a rosette of three fronds was added to each flask. One flask each was supplemented with solvent as control and for paraquat as standards growth inhibitor. All flasks were plugged with cotton and kept in the growth cabinet for 7 days. The number of fronds per flask were counted and recorded on day seven.

$$\text{Growth inhibition } (\%) = 100 - \frac{\text{No. of frond in test}}{\text{No. of frond in control}} \times 100$$

Leishmanicidal assay

Leishmania major (DESTO) promastigotes were cultured at 22-25°C in RPMI-1640 (Sigma). The medium was supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS). Promastigote culture in the logarithmic phase of growth was centrifuged at 2000 rpm for 10 min, and washed with saline three times in the same condition. Parasites were diluted with fresh culture medium to a final density of 106 cells /ml. In a 96-well microtiter plate, 180 ml of medium was added in first row and 100/ml of medium was added in others wells (Saeed

et al., 2010). Product (20/ml) was added in medium and serially diluted. 100 ml of parasite culture was added in all wells. One row was used for control (DMSO) which received medium while one for standard drugs (Amphotericin B, Pantamidine). The plate was incubated at 21-22°C for 72 h and the numbers of surviving parasites were counted microscopically in Neubauer chamber. Results are the replicates of three different experiments. The 50% inhibitory concentrations (IC_{50}) were calculated by a Windows based EZ-Fit 5.03 Perrella Scientific Software.

Brine shrimp cytotoxic assay

Brine shrimp cytotoxic assay was employed (Khan *et al*., 2011b) to determine the cytotoxic of Joshanda decoction. In this method, test samples were prepared in respective solvents in the concentrations of 10, 100, and 1000 µg/ml. Brine shrimp (*Artemia salina* Leach) nauplii were hatched in a specific tank at room temperature. From stock solutions, 5, 50 and 500 µg/ml was injected into 9 vials (3 vials for each dilution). Each vial contained ten shrimps and 5 ml of brine solution. Dry yeast suspension as their food was added to vials; incubated for 24 h under illumination. For activity determination, the live nauplii were counted using a 3 x magnifying glass and the deaths (%) at each concentration was noted. The resulting data were processed by using Finney programme on a simple computer to estimate LD_{50} values.

Phytochemical tests

The decoction of Joshanda was subjected to different phytochemical test like saponin, alkaloids, phenols, sterols, flavonoids, tannins (Khan *et al*., 2011c).

RESULTS

The results of antibacterial activity of the decoction of Joshanda of paper disc and agar well diffusion methods are depicted in table 1 and 2 respectively. The activity against Gram Positive tested pathogens was very prominent, similar to standard drug, Erythromycin. While the Gram Negative pathogens were not susceptible except *K. pneumoniae.* Notably, both the methods were equally responsive against tested pathogens.

Effect of antifungal activity

As shown in table 3, the decoction of Joshanda did not show any sensitivity against the only fungus used in test i.e. Candida.

Table 3: Antifungal activity of decoction of Joshanda against *Candida albicans*

Candida albicans	Activity (Zone of Inhibition) in ml			
	Disc		Well	
	A	B	A	B
	0.00	0.00	0.00	0.00

Data represent mean of three individual studies

Effect of phytotoxic activity

When tested against *L. acquinoctialis* Welv, the decoction of Joshanda showed prominent activity as shown in fig. 1. The effect was in concentration dependent manner with a maximum inhibition of 75% at the concentration of 500 mg/ml. While the standard drug used, paraquat exhibited more potent effect.

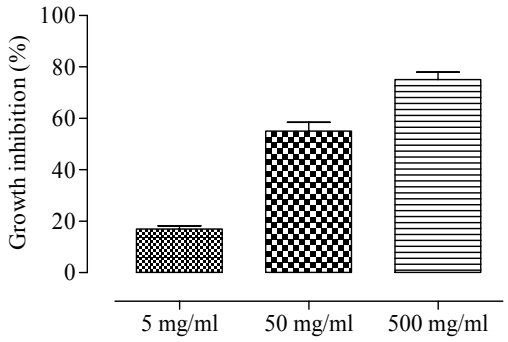

Fig. 1: Phytotoxic activity of the decoction of Joshanda against *Lemna acquinoctialis* Welv. Standard drug= Paraquat (3.142µg/ml). Data represent mean ± SEM of three individual studies.

Effect of leishmanicidal activity

The results of leishmanicidal activity of decoction of Joshanda are illustrated in table 4. It did not show any activity against *Leishmania major*.

Table 4: *In vitro* anti-leishmanial activity of the decoction of Joshanda against *Leishmania* major

Test organism	Drugs	IC_{50} (µg/ml)
Leishmania major (DESTO)	Decoction of Joshanda	> 100
	Amphotercin-B	0.50±0.02
	Pentamidine	2.56±0.02

Incubation period was 72 hrs at 22°C. Data represent mean ± SEM of three individual studies.

Effect of cytotoxic activity

The results of cytotoxic activity against brine shrimp (*Artemia salina* Leach) nauplii are shown in table 5. The test product had no cytotoxic effect while the standard drug used, showed prominent activity in the test

Table 5: *In vitro* brine shrimp cytotoxicity of the decoction of Joshanda against *Artemia salina* Leach

Test organism	Drugs	LD_{50} (µg/ml)
Artemia salina Leach	Decoction of Joshanda	NA
	Etoposide	07.4625

DISCUSSION

Herbal products are extensively available in different areas of Pakistan. The practice of traditional medicine (hikmath and homeopathy) is regulated by the Federal Government through Unani, Ayurvedic and Homeopathic (UAH) Practitioners Act, 1965. In this regard, the National Council of Tibb (NCT) and National Council for Homeopathy (NCH) were established as corporate bodies under section 3 of the Act. Their formation was aimed to encourage and popularize the traditional system of medicine in masses (Saeed *et al*., 2010; Saeed *at al*., 2011).

In the present study, the decoction showed marked activities against the Gram Positive pathogens mostly involved in upper respiratory tract diseases. *S. aureus*, *B. subtilis* and *B. cereus* have established role in the upper respiratory tract (Yablonsky, 1983; Pettigrew *et al*., 2008; Bottone, 2010). For that reason, our studies supported the uses of Joshanda in the upper respiratory infections. Further detail studies on the isolation of individual components could be helpful in the clinical application of it against such conditions. Interference of weeds obviously reduces the quality and quantity of agricultural crops and is responsible for huge economic losses all over the world. It is estimated in US alone that weeds cause a loss of at least 12% costing to nearly US$ 33 billion while the situation is more alarming in developing countries (Piment *et al*., 2001). Synthetic herbicides are extensively used for the control of weeds in agricultural sectors. However, various factors that restricted the use of synthetic herbicides include water and soil pollution, herbicide-resistant weed populations, herbicide residues and detrimental effects on non-target (Li *et al*., 2003). In recent times, more stress has been given to the natural allelelopathicchemicals (allelochemicals) from plants for weed control in crop production especially to cope with the problem of weed resistance. The decoction of Joshanda when tested in phytotoxic assay, it showed significant inhibitory effects on the growth of *L. acquinoctialis* Welv. It could therefore be a useful natural herbicidal.

In conclusion, the current studies supported the uses of Joshanda in the upper respiratory infections and as natural herbicidal. However, no effect was observed as leishmanicidal and cytotoxic. Further detail studies on the isolation of individual components could be helpful in the clinical application of it against respiratory pathogens and for the control weeds.

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